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Comment:

In this study, nerve cells of a *Helix pomatia* snail were subjected to a treatment with a non-ionizing, non-thermic electromagnetic field. Snails were chosen as the subject of the experiment due to their small number of neurons that are easily accessible. The central nervous system of 3-4 year old snails was removed. Nerve cells with a resting potential that remained unchanged during the 40 minute preliminary stage were chosen, and were exposed to weak HF magnetic fields for 10 minutes. After exposure, the membrane potentials of nerve cells were almost always hyperpolarized.

Hyperpolarization refers to an increase in the membrane potential. Nerve cells with a stronger, more negative resting potential were slightly hyperpolarized, while cells with a weaker resting potential were strongly hyperpolarized. The hyperpolarization ranged from 2 mV to 13 mV. After the membrane potential of the cells stabilized following the first exposure, a second exposure caused no further hyperpolarization. The hyperpolarization usually occurred in the last minute of the exposure, or 1-10 minutes after it ended.

Further research is needed to identify the mechanisms through which weak HF electromagnetic fields cause a change in the bioelectricity of cells. One possible explanation is that the ion channels within the cell membranes are altered, which would effect the membrane's permeability and resistance.

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Influence of weak non-thermic high-frequency electromagnetic fields on the membrane potential of nerve cells *

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Abstract

Nerve cells of the snail *Helix pomatia* were subjected to high-frequency (159 MHz, 8.3 Hz modulated), non-thermic (maximum flux density 124 μ T) electromagnetic fields. The effect of the fields on the membrane potential of various nerve cells was investigated. It was observed that short and unique Befeldungen¹ led to an alteration of the membrane potential of the neurons examined. The alteration almost always expressed itself as a long-term hyperpolarization of the resting potential. A clear connection between the negativity of the membrane potential of a nerve cell before the Befeldung and the strength of the hyperpolarization caused by the Befeldung was seen. As well as this effect, an alteration in the threshold of excitation of befeldet cells could be measured.

INTRODUCTION

The influences of so-called non-thermic high-frequency (HF) electromagnetic fields (EMF) on biological systems are becoming a greater part of public interest. The fields are being made use of more and more extensively for commercial therapy apparatus. This explains the discussion about their medical importance and their possible effects on the environment. It was against this background that the effects of weak ac magnetic fields began to become the object of numerous research projects [1]. In the meantime, a number of clinical findings seem to

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¹ Definition: Befeldung is treatment with a weak (non-ionizing, non-thermic) electromagnetic field.

confirm that there are effects which can be used therapeutically. Not only the unknown effect mechanisms, but also the lack of knowledge about the best therapeutic parameters of the fields used (intensity, frequency, modulation) are against sensible use. Also with regard to work and environmental risks, greater knowledge of the interactions between EMF and biological systems is necessary at all costs. This is of special importance because standards for the protection of mankind in the future could be formulated not only from an energy point of view [2]. It can be seen that the opinion of many researchers (frequently expressed up to now) that the effect of EMF can only be substantiated energetically must now be reconsidered in the wake of more modern knowledge. For investigations of the effect of weak electromagnetic fields in this study, the central nervous system (CNS) of the vineyard snail is used. The advantages of using a snail as the object of investigation can be found in the comparatively small number of neurons (approximately 50 000), their easy accessibility, their specific size (up to 150 μm) and their robustness against interventions in the nervous system (e.g. preparation). Further, it is a well-known fact that the fundamental functions of nerve cells are practically identical in the various groups of animals, both from a metabolic and an electrophysical point of view (signal generation, conduction and transmission). A great deal of correspondence can be found especially in molluscs and the neurons of vertebrates (Hodgkin, Huxley 1952). A principal transferability of the effects of electromagnetic fields observed in the neurons of snails to human nerve cells can thus be presupposed. The neurons of molluscs make it possible to recognize not only short-term, brief effects, but also long-term or irreversible ones, as they can be measured intracellularly for a number of hours and even days, as opposed to the nerve cells of vertebrates. *Helix pomatia* is thus a suitable model organism for the investigation of neuronal questions. In addition, experiments with vertebrate cell cultures and preparations of brain slices will be carried out in our laboratory in the near future.

The first investigations of the nerve cells of *Helix pomatia* and their bioelectricity under the effects of weak HF magnetic fields (150 MHz, 8.3 Hz, LF pulsed) gave interesting results [3]. The parameter which was predominantly investigated, the membrane potential, showed clear hyperpolarization of the nerve cells. These results were confirmed, completed and extended in further examinations. Perhaps they can form the basis for the solution to the problem of cell-physiological mechanisms of the athermic effect of HF-EM fields.

METHODS

For the experiments, adult vineyard snails (3–4 years old) of the species *Helix pomatia* (Gastropoda, Pulmonata) were used. The CNS of the animals was removed without the use of medicines. The CNS consists of an oesophageal ganglion with cerebral and suboesophageal ganglia. For intracellular measurements, only giant nerve cells of the suboesophageal ganglion were used (the area marked by a

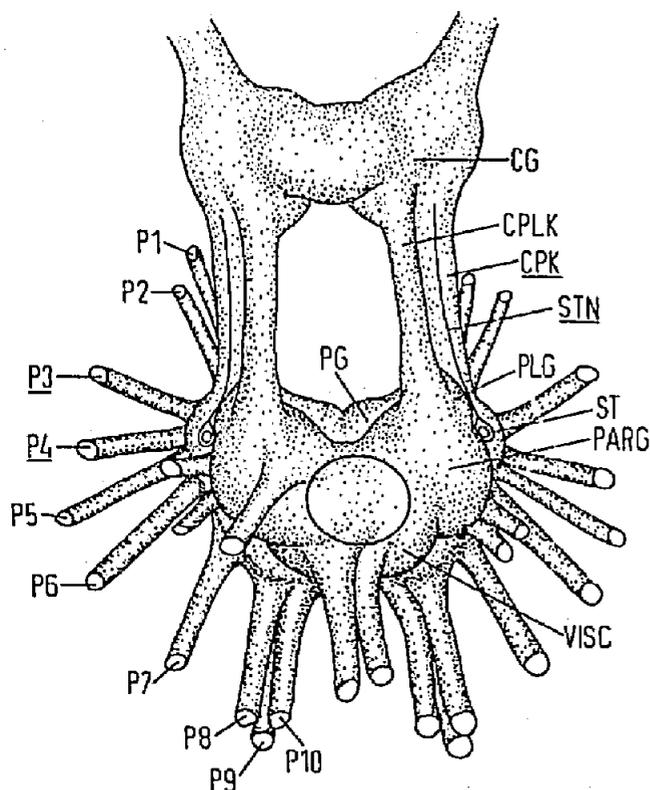


Fig. 1. CNS of *Helix pomatia* with intact connective tissue. CG: cerebral ganglion; CPLK: cerebro-pleural connective; CPK: cerebro-pedal connective; P1-P10: pedal nerves; PARG: parietal ganglion; PD: pedal ganglion; PLG: pleural ganglion; ST: statocyst; STN: stato nerve; VISC: visceral ganglion. The neurons measured are those in the circle.

circle in Fig. 1). The cell diameter was approximately $100 \mu\text{m}$. Selection of the measured cells depended on their position relative to the electrode.

Measurements of the membrane potential (MP) of living cells, which were not obviously stimulated, by use of suitable measurement systems always show characteristic voltage values, called resting potentials (RP), which can remain constant for a long time as a rule. In all resting potentials, the membrane of the nerve cell on the inside has a negative load compared with the outside. In this study, this resting potential was the predominantly investigated parameter, in order to establish the effects of high-frequency electromagnetic fields on nerve cells.

Alterations of the membrane potential occur in physiological excitations or artificial electrical stimulation of the cell. An increase in the membrane potential, i.e. increased negativization of the inside, is called hyperpolarization, a reduction in the potential being depolarization. The membrane potential of nerve cells mainly arises through:

- (1) a semi-permeable cell membrane; or
- (2) an uneven distribution of ions between the intracellular and the extracellular fluid, which is produced, inter alia, by energy-consuming transport processes.

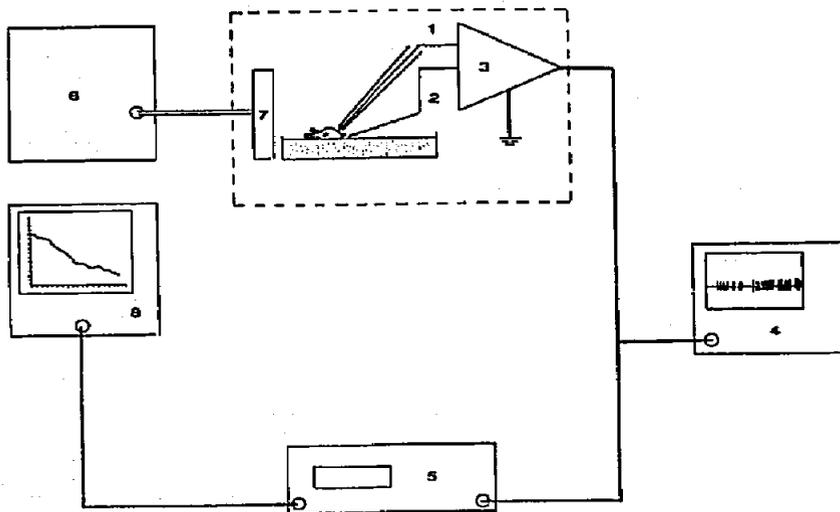


Fig. 2. Experimental set-up for intracellular measurements. (1) Signal connection; (2) signal-ground connection; (3) intercellular preamplifier; (4) oscilloscope; (5) DAT recorder; (6) Mega-Wave 150/1; (7) EMF transmitter; (8) off-line computer.

Resting potential and other bioelectrical signals (AP, EPSP, IPSP) are recorded by measurement probes (electrodes). For intracellular measurements, these are fine glass capillaries with tip openings of around $1 \mu\text{m}$, filled with a 3 M KCl solution. With the help of such micro-electrodes and their conductive connection (Ag-AgCl wire) and a metal signal-ground connection in the Ringer solution in which the objects to be measured can be found, and also measuring instruments with a high electrical resistance, the membrane polarity of a nerve cell can be measured and portrayed. For reasons to be found in the physics of high-frequency electromagnetic fields, there should be no metal parts in the area of the effects of the electromagnetic fields used. In the area of the magnetic field, we therefore managed without metal conductors, especially in the measurement area, in order to avoid physical interactions which could falsify the measurement or possibly even make it useless. These demands were fulfilled by the development of a new, modified intracellular measuring method.

Measurements of the membrane potential in the magnetic field were made possible by replacing the chlorinated silver wire (signal and signal-ground connection) inside the field with a 3 M HCl-agar-agar bridge in thin plastic hoses. The agar bridges only pass into the silver wires, which conduct the measured signals to the amplifier (Fig. 2), at a distance which basically rules out an influence of the applied magnetic field. This method makes intracellular measurement of nerve cells in high-frequency electromagnetic fields possible. In order to avoid further possible measurement artefacts caused by an unfavourable measurement geometry, the signal and signal-ground connections were arranged more or less in the direction of the spread of the electromagnetic field (Fig. 3).

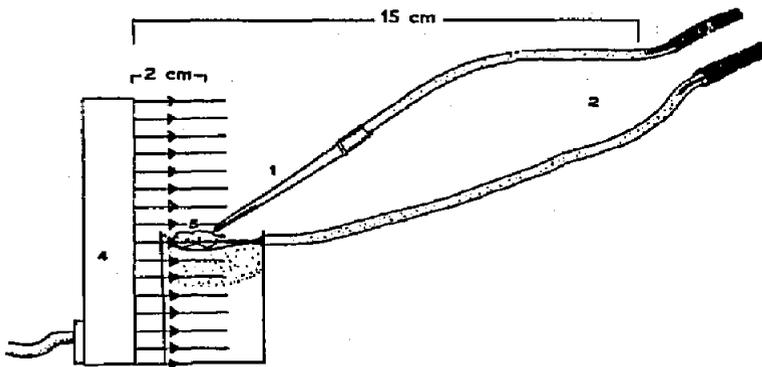


Fig. 3. Modified intracellular measurement. (1) Glass signal connection (3 M KCl); (2) KCl-agar bridges (3 M KCl); (3) connection of agar bridges and Ag-AgCl wire; (4) EMF transmitter; (5) ganglion.

Cells with high spontaneous activity were not used in the experiment. Cells whose membrane potential showed many EPSP (excitatory postsynaptic potentials) and IPSP (inhibitory postsynaptic potentials) were also not used for the evaluation. Basically, only nerve cells with a resting potential which remained unchanged (2 mV) for at least 40 min (preliminary stage) were used in the evaluation. Following the experiment, no identification of the penetrated cell was carried out by neuro-anatomic staining or functional checks.

The measurement was always carried out in the following sequence: 40 min preliminary phase, 40 min run-up, 10 min Befeldung, 40 min run-down, 40 min subsequent phase. The fields used had the following characteristics: carrier frequency: 150 MHz; modulation frequency; 8.3 Hz; form of modulation: needle impulse; magnetic flux density; $124 \mu\text{T}$ 50% (2 cm from the transmitter, Institute for Experimental Physics, Free University of Berlin); time of application: 10 min.

The data recorded by a DAT recorder (48 kHz scan frequency) were read into an off-line computer. The computer evaluation was carried out on a Commodore PC by means of an analysis program developed in our department. This program works with a scan frequency of 32 kHz. The mean values are taken from ten measured values. This averaging is carried out until a mean value for 1 min of measuring is produced. The program recognizes technical interference, action potentials and sudden slight alterations of the membrane potential. These are faded out with slight run-up or run-down times (1 s) and are not used in the computation of the mean value. The times faded out are displayed and should not be longer than 1 min in an experimental period of 90–140 min.

Measurement of the threshold of excitation

The nerve cells were excited intracellularly just above the threshold by signal connections with rectangular impulses in accordance with the standard method. Each cell was excited ten times at intervals of 10 s. This took place 10 min before the Befeldung, during the Befeldung, 10 min after this and, for a final time, 1 h after the end of the Befeldung.

RESULTS

Measurement of the resting potential of the so-called quiet cell

The single Befeldung of a nerve cell almost always resulted in the resting potential being more or less strongly hyperpolarized. The hyperpolarization sometimes started shortly after the start of the Befeldung. However, hyperpolarization normally occurred only in the last minute of the Befeldung or, quite frequently, only 1–10 min after the end of the Befeldung (Fig. 4). The nerve cell somata measured had membrane potentials between -23 and -68 mV. The hyperpolarization in these cases was -2 and -13 mV (cf. Table 1).

The strength of the evoked hyperpolarizations is closely connected with the membrane potentials of the cells. Nerve cells with highly negative RP (e.g. -68 mV) were only slightly hyperpolarized by the Befeldung (approximately -2 mV). On the other hand, cells with a weak RP (e.g. -23 mV) were strongly hyperpolarized (e.g. 13 mV) (Fig. 5). Thus, there is a connection between the value of a resting potential set by the cell and its hyperpolarization caused by the field.

The hyperpolarization also lasted for a number of hours. As the preparation, as described in the Methods section, was not in a nutrition solution but in a blood replacement solution, it was not possible to establish under these experimental conditions whether the depolarization of the membrane potential, which sometimes only occurred after several hours, was due to a drop in the effect of the Befeldung or to physiological reactions of the cell to a lack of supply (Fig. 6).

Measurement of the hyperpolarized resting potential after a second Befeldung

When the hyperpolarization of a befeldet cell had come to a standstill, i.e. the membrane potentials had taken on a stable value, a second Befeldung was carried

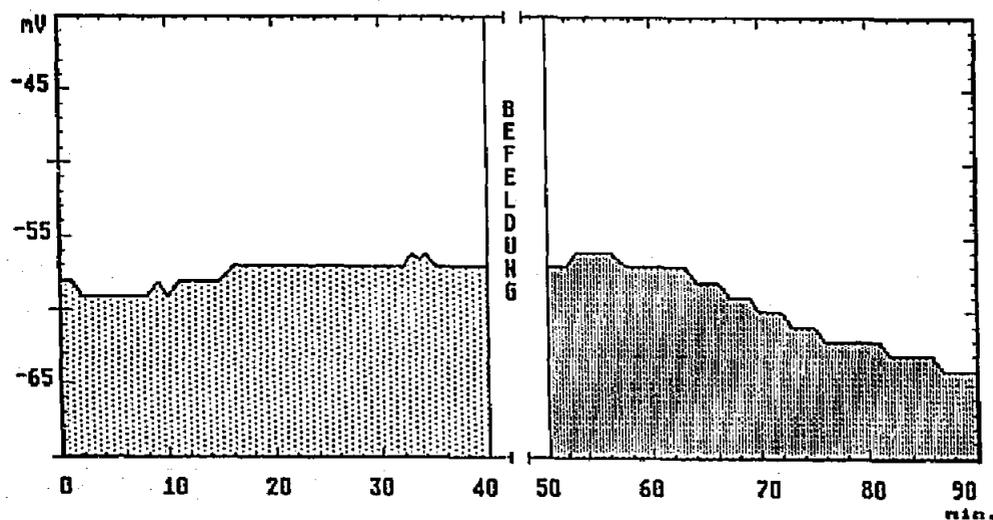


Fig. 4. Single Befeldung after one Befeldung. The resting potential of the measured hyperpolarizations was approximately 7 mV.

TABLE 1

Dependence of the hyperpolarization values on the membrane potential of some nerve cells. Neurons with highly negative membrane potentials hyperpolarize slightly after one Befeldung. Neurons with weakly negative membrane potentials hyperpolarize strongly after one Befeldung

Resting potential start value/mV	Resting potential after 1st Befeldung/mV	Hyperpolarization/mV
-68	-70	2
-67	-68	1
-67	-70	3
-66	-69	3
-63	-68	5
-62	-65	3
-58	-62	4
-57	-61	4
-56	-62	5
-46	-55	9
-40	-47	7
-39	-45	6
-39	-47	8
-38	-47	9
-28	-38	10
-27	-37	10
-25	-36	11
-24	-37	13

out on a number of occasions. The newly set membrane potential did not hyperpolarize further. A third Befeldung also had no measurable effect on the development of potential (Fig. 7).

Befeldung of non-quiet cells

(a) Alongside quiet cells, other nerve cells were also included in the experiment. Neurons were befeldet, the resting potential of which was not stable but had a depolarizing tendency. This tendency was stopped by the Befeldung and, in some cases, even reversed (Fig. 8).

(b) Some cells, which were possibly excessively damaged in the penetration of the signal connection, showed strong depolarization. The Befeldung did not, as in case (a), achieve a drop in the depolarization, but the exact opposite (Fig. 9). The depolarization was clearly increased and practically led to potential compensation after a short time (approximately 1 h).

Measurement of the threshold of excitation

Naturally, it was interesting to see how the threshold of excitation of a nerve cell would behave if its membrane potential was hyperpolarized by Befeldung.

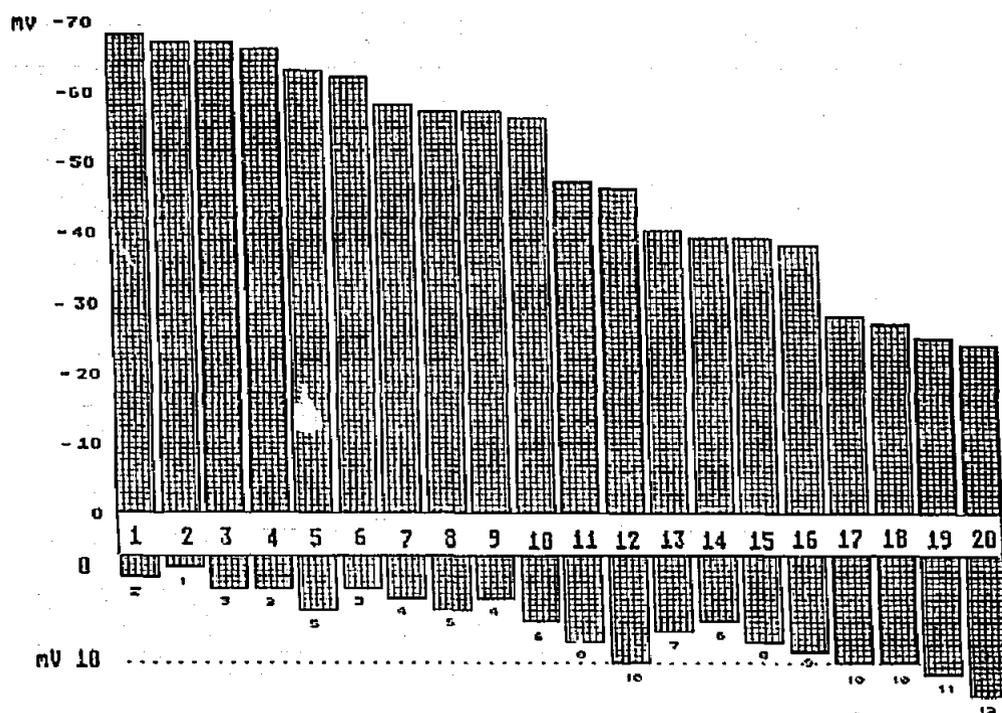


Fig. 5. Hyperpolarizations after one Befeldung as a function of the initial membrane potential. Upper line: membrane potentials of various cells before the Befeldung; lower line: hyperpolarizations of these cells after one Befeldung.

Unfortunately, there are few results concerning this question. Yet these are very interesting, for which reason they are portrayed here (Table 2). Unfortunately, it has only been possible to carry out the experiment three times up to now, the result being the same each time. The electrical intracellular stimulations, to which a reaction came with action potentials (AP) in about 83% of the cases in the run-up, which brought about a reaction of 36.6% during the Befeldung. Ten

TABLE 2

Measurement of the threshold of excitation before, during and after a Befeldung (three different nerve cells). Evoked action potentials (eAP) caused by electrical rectangular stimuli before, during, 10 min and 1 h after a Befeldung

RP/ mV	Before Befeldung eAP	During Befeldung eAP	10 min after Befeldung eAP	1 h after Befeldung eAP	Hyperpolarization mV
-58	28	12	17	22	-5
-40	27	10	15	20	-7
-56	28	11	16	22	-6
eAP total =	83%	36.6%	53.3%	71%	

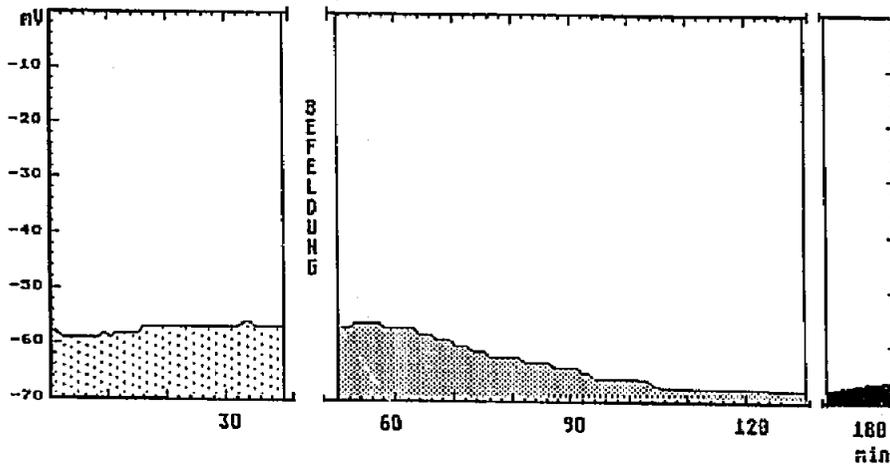


Fig. 6. Alterations of the membrane potential of a nerve cell after one hyperpolarization. Approximately 90 min after the end of the Befeldung, the hyperpolarization of the membrane potential due to the field has finished in this case. After a plateau phase, slow depolarization starts about 3 h after the end of the Befeldung.

minutes after the end of the Befeldung, this figure rose to 53.3% again, even rising to 71% after 1 h. However, it must be mentioned that the cells used in these experiments had a relatively strong negative resting potential, which means that the hyperpolarization was not very strong.

Measurement of artefacts

The problem with electrophysiological measurement in electromagnetic fields is to cover all possible artefacts which may influence the nerve cell wire of the

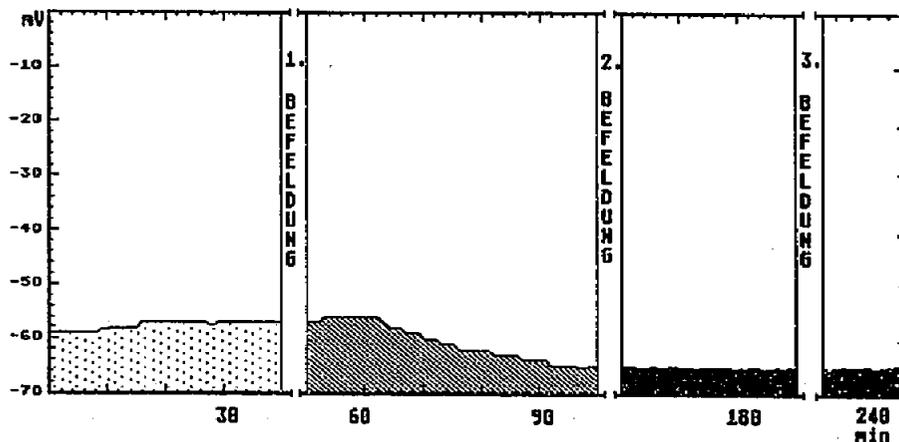


Fig. 7. Alteration of the membrane potential of a nerve cell with three Befeldungen. Only the first Befeldung leads to hyperpolarization of the membrane potential. Befeldung 2 and 3 have no effect.

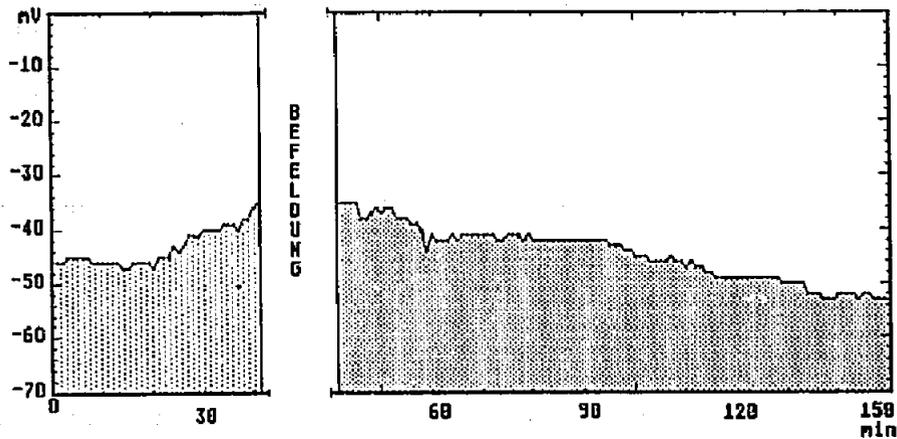


Fig. 8. Alteration of the membrane potential of a non-quiet cell after a single Befeldung. The low depolarization of the membrane potential of a nerve cell is stopped after one Befeldung and then even brought to hyperpolarization.

recording electrode. There are some physical phenomena (magnetic induction, radio aerial effect, influence, skin effect, etc.) which have properties masking the ability to influence the measurements. In order to escape from magnetic voltage induction we interrupted the measuring loop during the electromagnetic Befeldung. The disadvantage of this type of measurement, however, is that the neurons (RP) cannot actually be tested during EMF stimulation. The electrical influence was tested experimentally and we could not see any artefact. Other effects were calculated (Institute of Experimental Physics, FU, Berlin) but there was no indication of any electrical or other influence on the measuring system.

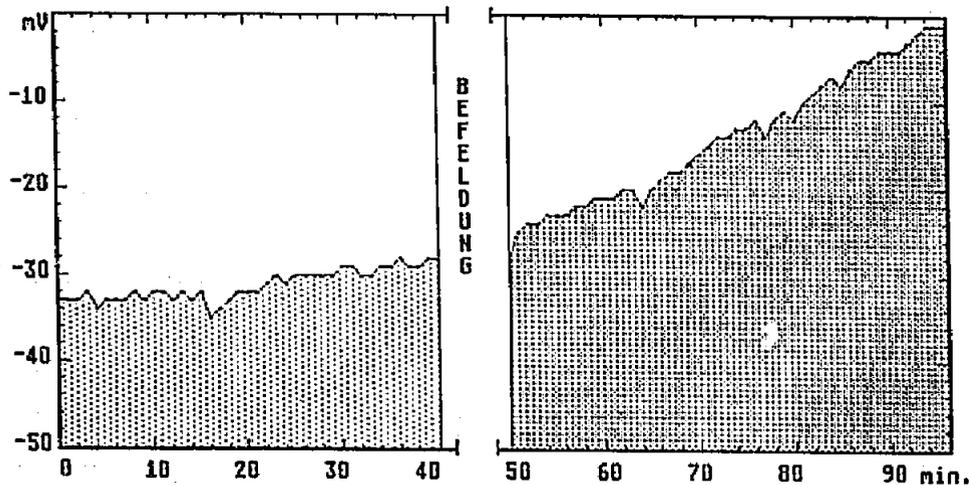


Fig. 9. Alteration of the membrane potential of a nerve cell depolarizing due to injury, after one Befeldung. The depolarization of the cells is strongly accelerated after the Befeldung.

Naturally, Befeldung was also carried out on cells which had completely collapsed physiologically (almost complete compensation of potential, no EPSP, IPSP or AP, and no electrical excitability). In the Befeldung of these cells, there was no alteration of the potential measured. In the experiments with quiet cells, a relatively uniform tendency towards hyperpolarization was seen. The relationship between the resting potential and the strength of the hyperpolarization to be attributed to the Befeldung (high resting potential — weak hyperpolarization; low resting potential — strong hyperpolarization) corresponds to the quantitative relationship between the concentration ratio and the equilibrium potential on nerve cell membranes (Nernst's equation). This is also why repeated Befeldung in the experiment did not lead to any further hyperpolarizations.

DISCUSSION

The results of the Befeldung of non-quiet cells match the above-mentioned attempts at explanation. The end of depolarization tendencies corresponds to that of the hyperpolarization and could be subject to the same effect mechanisms. The measurement of highly damaged nerve cells is different. The acceleration of the potential compensation could be substantiated by the fact that the mechanisms which led to the hyperpolarization of quiet cells can no longer work effectively. Nevertheless, they could possibly represent a process which consumes a large amount of energy. This energy consumption could lead to a quick collapse of the bioelectricity of the nerve cell.

The measurement of the threshold of excitation of a nerve cell showed quite special results. An interpretation can only be made very reticently here. Obviously, the strongest effect of the electromagnetic fields during the Befeldung is that it causes a drop in the evoked potentials to 36%. The reaction to electrical stimuli increases again after the Befeldung. This appears to contradict the results obtained up to now, as the excitability is at its lowest at the moment when the hyperpolarization caused by the field is also at its lowest, sometimes hardly measurable. The excitability increases again with the amount of hyperpolarization or with the drop in the Befeldung. All the findings measured indicate that various effect mechanisms exist in the field effect on nerve cells.

More precise and more numerous measurements in our institute, inter alia on brain sclices and nerve cell cultures, and examinations on ion channels will produce further results in the near future and thus possibly contribute to the formulation of a general theory of effects. As there has been frequent speculation in the medical literature in the recent past concerning the effects of weak electromagnetic fields in the immune system of man, investigations are currently being carried out in our laboratory on neurosecretory nerve cells on molluscs (*Lymnaea stagnalis*), the aim being to examine the field effects referred to in this paper.

Owing to the change in methods in the intracellular measurement of nerve cells, it was possible for the first time to measure the membrane potential free of

artefacts during and after the Befeldung with weak HF electromagnetic fields. Thus, the effects which the fields used had on the bioelectricity of the nerve cells (clear hyperpolarization) were proven. These results were especially important because it was possible to show clear influences on the electrical properties of nerve cells, despite the use of field strengths below the mean energy of molecular thermal motion.

As thermic effects through the fields used are ruled out [4], one or more different effects must cause the alteration of the bioelectricity of the cells investigated.

As the membrane potential is basically a biophysical phenomenon, which can be explained by the specific properties of the cell membrane, it would be possible to imagine effect mechanisms which have an effect on elements of the membrane, e.g. various protein structures, on the basic structure of the membrane (phospholipid double layer) in its interpretation as a liquid crystal or membrane condenser or on the energetics forming the basis of the active transport mechanisms. The alterations of the ion channels which result from this, and therefore also of the permeability and the resistance of the cell membrane, could explain the measured alterations of the bioelectricity of the nerve cell.

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